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DRY ICE FIXATION OF MYOFIBRILS FOR SCANNING ELECTRON MICROSCOPY

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ABSTRACT. **A rapid method of fixation of myofibrils using dry ice is reported. A glass slide or coverslip containing a drop of glutaraldehyde-fixed suspension of myofibrils is placed on dry ice causing the myofibrils to adhere to the glass surface. The specimens are then dehydrated through the alcohols, air dried and metal coated. This technique gives the myofibrils a corrugated appearance under the scanning electron microscope corresponding to the sarcomere banding.**

A great deal of work has been done with the scanning electron microscope (SEM) to analyze muscle fibers. Boyde and Williams (1968), Haggis (1970), Schaller and Powrie (1971), Stanley and Geissinger (1972), Pachter (1973) and McCallister *et al.* (1974) are a few of the investigators who have studied either cardiac or skeletal muscle fibers. The preparative techniques they employed either singly or in combination with others included air drying, freeze drying, freeze fracturing, critical point drying and ion etching.

The above methods were quite satisfactory for studying strips, blocks or thin sections of muscle fibers; however, for our research on the effects of tenderizing enzymes on the A, I and Z bands of bovine myofibrils (Robbins and Cohen, in press) a method for observing changes in the bands of individual myofibrils was of major importance. Because of the great number of samples we used (several hundred), such time-consuming procedures as critical point drying and freeze drying were ruled out; however, to check on distortion and shrinkage selected samples of myofibrils were critical point dried and compared with other myofibrils examined by phase contrast microscopy, and with those prepared by the technique described in this paper.

A quick and simple technique similar to one for preparing certain tissues for chromosome analysis (Cohen and Roth 1970) was therefore developed for the observation of isolated bovine semimembranosus myofibrils by scanning electron microscopy.

PROCEDURE

1. Prepare myofibril suspension as described by Perry and Grey (1956) and Perry and Corsi (1958).
2. Fix 1 ml of myofibril suspension in 10 ml 2.5% glutaraldehyde in KCl-borate buffer pH 7.1. Mix thoroughly for 1 min.
3. Using a Pasteur pipet, place one drop of myofibril suspension on a clean glass slide or coverslip.
4. Put a coverslip over the drop to spread the suspension evenly and blot the excess fluid with bibulous paper.
5. Place the glass slide on dry ice, leaving the coverslip on. If two coverslips are used, separate them by pulling in opposite directions and place both coverslips face up on the dry ice.

6. After 5 minutes, remove the glass slide from the dry ice, flip the coverslip off with a single-edged razor blade and put the slide immediately in 70% alcohol for 1 min at room temperature. If coverslips are used, remove tham from the dry ice and treat them in the same manner.

7. Transfer slide or coverslips to 95% and 100% alcohol for 1 min each, then air dry.

8. Using a diamond pencil cut out the section of the glass slide with the myofibrils and mount it on an SEM specimen stub with silver paint. Mount coverslips in the same manner.

9. After evaporation of gold-palladium onto the surface of the glass slide or coverslip, it is ready for insertion into the SEM.

Results and Discussion

Mammalian skeletal muscle myofibrils are irregular polygons which, in cross section, appear approximately circular and are therefore considered to be right circular cylinders for purposes of measuring volume and area (Eisenberg and Kuda 1975). Bovine semimembranosus myofibrils are typically mammalian in that they are enmeshed in the sarcoplasmic reticulum and are, as bundles composing muscle fibers, enclosed within the sarcolemma. Both sarcoplasmic reticulum and sarcolemma obscure

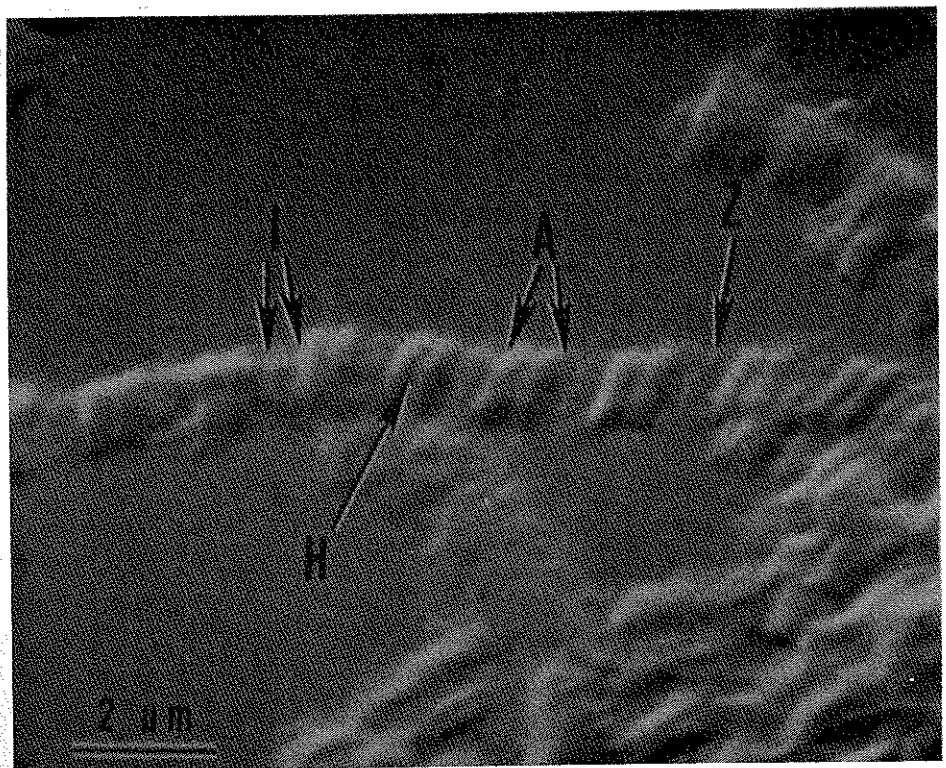

Fig. 1. Isolated myofibril. The H band appears as a depression in the center of the A band, whereas the Z band is elevated slightly in the center of the I band. The bar equals 2 µm. Coates and Welter Field Emission Scanning Electron Microscope Model 100-2. 10,000×.

the bands of the myofibrils; however, the maceration used to produce the myofibril suspension of Perry and coworkers frees the myofibrils from their connective tissue covering.

Pressure on the coverslip and surface tension effects cause flattening of the cylindrical myofibrils, revealing under the SEM a corrugated appearance (Fig. 1). This appearance can be seen in Stanley and Geissinger's (1972) paper on porcine psoas muscle, but because of the number of connected myofibrils, the three-dimensional quality is not as dramatic as with the isolated myofibril.

During contraction and relaxation, the size and shape of the bands may change. There is some shrinkage caused by the preparative technique described here. In twenty samples compared by phase microscopy before and after critical point drying and before and after preparation by the method described above, it was found that the maximum excess shrinkage due to the dry ice fixation procedure was about 20%, with the average about 14%.

Since our research interest is in the comparative effects of enzymes on the A, I and Z bands, the technique described in this paper was quite satisfactory. SEM examination of individual myofibrils, therefore, provides a general topographical view which, when used with light and transmission electron microscopy, allows for a better understanding of myofibril morphology.

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